The Role of Developmental Genetics in Understanding Homology and Morphological Evolution in Plants

Int. J. Plant Sci. 168(1):61–72. 2007.

1058-5893/2007/16801-0004$15.00

THE ROLE OF DEVELOPMENTAL GENETICS IN UNDERSTANDING HOMOLOGY AND MORPHOLOGICAL EVOLUTION IN PLANTS

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Homology assessments are critical to comparative biological studies. Although gene expression data have been proposed as instrumental for defining homologous relationships, several lines of evidence suggest that this type of data can be misleading if used in isolation. The correspondence between the homology of genes and that of structures is not simple, and conclusions can be derived only after careful examination of all available data. For instance, the MADS-box gene family is one of the best-studied families of transcription factors, and it provides several examples of dissociation between genetic and morphological homology. In this regard, we examine the role of *APETALA3* and *PISTILLATA* homologs in the development of petaloid organs, a feature thought to have originated multiple times. We also consider the role of members of the *AGAMOUS* subfamily in the development of the pistil, a character that originated only once. Additionally, we discuss how serial homology makes gene cooption a very common phenomenon in plants. In spite of the multiple cases of this type of dissociation, comparative developmental genetics can yield other types of information that help assess homologies. Furthermore, comparative gene expression studies provide useful data for dissecting the origin of morphological innovations and are, therefore, key to understanding character evolution. Finally, we provide some guidelines for the critical examination of comparative gene expression data in the context of studying morphological innovations.

Keywords: plant developmental evolution, homology, MADS-box genes, YABBY genes, orthology.

A Definition of Homology

The concept of homology was born in the realm of comparative anatomy (Owen 1848). Later, homology assumed a central role in evolutionary theory as homologous features were perceived as being derived from a common ancestor (reviewed in Panchen 1999). The concept of homology has provoked many controversies and the production a large body of literature from both a philosophical and a methodological perspective (e.g., Patterson 1982; Roth 1984; Stevens 1984; Wagner 1989; Sattler 1994). This controversy was further complicated by the arrival of gene expression data (Bolker and Raff 1996; Wray and Abouheif 1998; Wray 1999). The many definitions of homology emphasize the importance of either similarity or ancestry (reviewed in Donoghue 1992; Laubichler 2000; Brigandt 2003). Here we adopt the homology concept of Van Valen (1982, p. 305), who defined homology as the "correspondence caused by a continuity of information." In terms of our current evolutionary standpoint, the information is primarily genetic, and the continuity is provided by genealogy (Roth 1988). This general concept of continuity of information allows us to account for both historical and serial homology. Historical, or taxic, homology refers to features present in two or more organisms that are derived from corresponding features in their common ancestor (Mayr 1982). Serial, or transformational, homology refers to the correspondence of features within the same individual (Roth 1984).

The concept of serial homology is particularly important in plants because they are masters of modularity. The basic building block of the plant body is the phytomer (fig. 1), composed of a lateral determinate organ, an axillary meristem, and an internode (Bell 1991). These modules are produced repeatedly during the construction of the plant body, a continuous process that starts only during embryogenesis (Walbot 1996). Diversity in plant morphology is generated by varying many aspects of the phytomer, the most important of which is the apical meristem identity program. Apical meristem identity, in turn, influences a wide array of characteristics, including the identity programs expressed in the lateral determinate organs, the activity of the axillary meristems, the relative positions of the lateral organs (referred to as phyllotaxy), and the elongation of the internodes (Steeves and Sussex 1989).

The complete modularity of the plant body means that much of plant diversity is derived from homeosis—changing the positional deployment of a wide array of identity programs, such as those controlling meristem or floral organ identity. According to Sattler (1994, p. 441), the common occurrence of homeosis in plants "undermines two widely accepted tenets of comparative morphology: (1) the importance of relative position in the establishment of correspondence or (potential) homology, and (2) the assumption that all correspondence between structures must be 1 : 1 correspondences." For instance,

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Manuscript received September 2005; revised manuscript received January 2006.

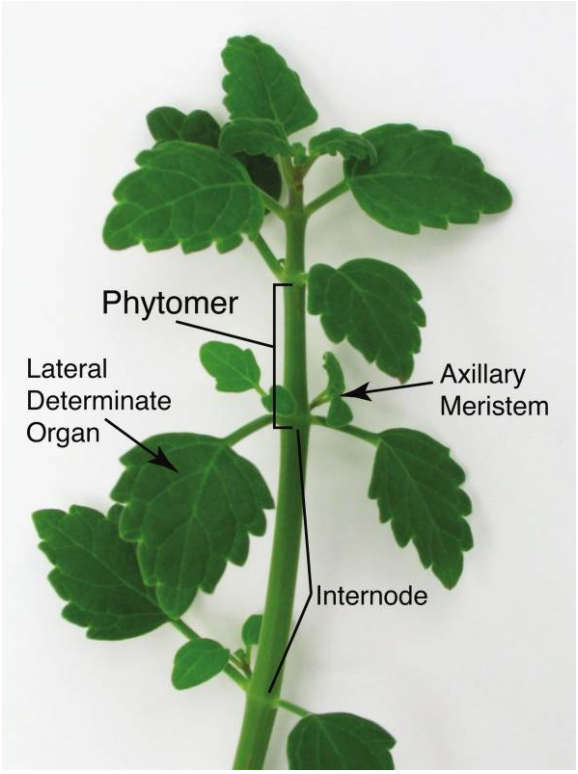

Fig. 1 Photo illustrating a phytomer composed of a determinate lateral organ, an axillary meristem, and an internode.

although stamens usually are positioned between the sterile organs and the carpels, they can also be located in the outermost whorl of the flower (*Eupomatia*; Endress 2003; Kim et al. 2005*a*) or internal to the carpels (*Lacandonia*; Martinez and Ramos 1989). Similarly, stamens vary enormously in number between species (Endress 1994) and are rarely present as morphological singulars (rare exceptions include orchids such as *Calypso bulbosa* var. americana [R.Br.] Luer, which produces a single flower with a single functional stamen; Luer 1975). Despite this, the homology of stamens across the angiosperms is unquestioned (Takhtajan 1991). Thus, homology assessments in plants can be complicated by their high degree of modularity and the flexibility with which identity programs can be expressed. Baum and Donoghue (2002, p. 58) discuss this phenomenon in detail and point out that "if all the genes expressed in the petal of an ancestor came to be expressed in a position that was previously occupied by a sepal . . . , the resulting structure would share genetic identity with a petal while showing positional homology to a sepal." Alternative terms have been suggested in the literature for such conflicts, including homocracy (Nielsen and Martinez 2003), which is intended to describe structures that are organized through the expression of identical patterning genes. As more information concerning the genetic control of plant identity programs becomes available, it is increasingly apparent that homology assessment in plants requires explicit consideration of the dissociability that exists between position and identity (Albert et al. 1998; Baum and Donoghue 2002; Hawkins 2002).

Beyond the morphological, however, it is also important to remember that homology, as a biological property, can be examined at other hierarchical levels, particularly the homology of individual genes and genetic pathways (Bolker and Raff 1996; Abouheif 1997; Hall 1999). When we consider the evolutionary relationships among genes, we refer to genetic homology (Hall 1999). This can further be distinguished into orthology, the relationship between loci that have been inherited through the common descent of species, and paralogy, the relationship between loci that were derived from a gene duplication event (Fitch 1970). The next hierarchical level of homology reflects the inheritance of modular genetic pathways, which is sometimes referred to as process homology (Abouheif 1999; Gilbert and Bolker 2001). The correspondence among these hierarchical levels is not always direct, and there are many cases of evolutionary dissociation that have been widely discussed in the animal evo-devo literature (e.g., Bolker and Raff 1996; Abouheif 1997; Holland 1999; Wray 1999; Mindell and Meyer 2001). The relation between the homology of genes and morphology in plants is equally complex because of the common occurrence of gene duplications and spatial shifts in gene expression that decouple topological correspondence (position) from morphological similarity (identity) as evidence for the homology of morphological structures. In this review, we present examples to illustrate each of these issues in turn and thereby seek to make the case for the consideration of all these hierarchical levels in the context of botanical studies.

Diversification of a Homologous Identity Program through Gene Duplication: One-to-Many Comparisons

A common expectation is that homologous genes should be expressed in homologous structures (Roth 1984). Consistent with this supposition, early studies of the genetics of floral organ identity in the eudicot model species *Arabidopsis* and *Antirrhinum* (Carpenter and Coen 1990; Bowman et al. 1991) suggested that homologous genes function in a very similar manner in both of these species. Detailed characterization of mutants exhibiting homeotic transformations of floral organ identity served as the basis of the ABC model of flower development (Coen and Meyerowitz 1991). Under this model, sepal identity is encoded by the A function alone, petal identity by A + B, stamen identity by B + C, and carpel identity by C alone (fig. 2*A*). Mutants in each class exhibit homeotic transformations of organ identity in two adjacent whorls; for instance, B mutants have petals transformed into sepals and stamens into carpels (Bowman et al. 1989). The genes corresponding to these classes have been well characterized and in *Arabidopsis* are represented by *APETALA1* (*AP1*) and *APETALA2* (*AP2*) in the A class, *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) in the B class, and *AGAMOUS* (*AG*) in the C class (fig. 2*B*; Bowman et al. 1989, 1991). All but one of these genes are members of the pan-eukaryotic MADS-box family of transcription factors (reviewed in Theissen et al. 2000), the exception being *AP2*. Further characterization of the MADS-box family in *Arabidopsis* has led to the recognition of a fourth major class of organ identity genes, the E-class, or *SEPALLATA*, genes (*SEP1–4*), which facilitate the functions of the original organ identity loci

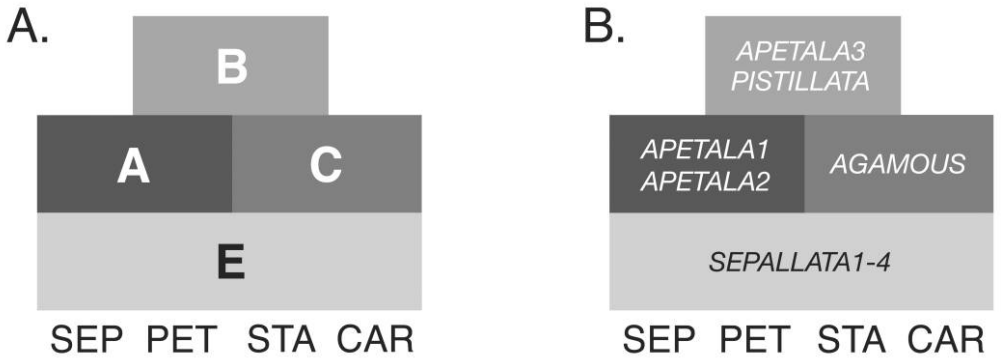

Fig. 2 *A*, Schematic representing the ABCE model for *Arabidopsis*. *B*, Same model as in *A*, showing the *Arabidopsis* genes corresponding to each class. *SEP* = sepal, *PET* = petal, *STA* = stamen, *CAR* = carpel.

(fig. 2*B*; Pelaz et al. 2000; Honma and Goto 2001; Ditta et al. 2004). The A-, B-, and C-class mutants from among the core eudicot model species show very similar morphologies (reviewed in Theissen and Saedler 1999). However, now that we have a more thorough understanding of MADS-box gene phylogeny, we see that the orthology of the genes and their functional evolution are not so simple. In particular, multiple intervening gene duplication events have resulted in a lack of one-to-one correspondence among the genetic systems of model species.

A good illustration of this phenomenon is the B-class homologs, which exhibit a dissociation of their petal and stamen identity functions in some taxa. The B-class genes of *Arabidopsis*, *AP3* and *PI*, and their respective orthologs in *Antirrhinum*, *DEFICIENS* (*DEF*) and *GLOBOSA* (*GLO*), represent two major lineages of the MADS-box gene family (fig. 3*A*; Kramer et al. 1998). Their gene products function as obligate heterodimers to promote both petal and stamen identity in developing flowers (Jack et al. 1994; McGonigle et al. 1996; Riechmann et al. 1996). The lineages themselves are closely related, having been derived from a gene duplication that predates the diversification of the angiosperms (Purugganan et al. 1995; Aoki et al. 2004; Kim et al. 2004; Stellari et al. 2004). Within the individual *AP3* and *PI* lineages, there have been many independent gene duplication events (Kramer et al. 1998, 2003; Tsai et al. 2004; Stellari et al. 2004; Aagaard et al. 2005). Perhaps most notably, the *AP3* lineage experienced duplication near the base of the core eudicots to give rise to the distinct paralogous eu*AP3* and *TM6* lineages (fig. 3*A*; Kramer et al. 1998). Both *AP3* itself and *DEF* are representatives of the eu*AP3* lineage, and no *TM6* ortholog has been characterized in the two primary model species. In contrast, the eudicot model *Petunia* retains both ancient paralogs: *PhTM6* and *PhDEF*, the genetic ortholog of *AP3* (fig. 3*A*; Kramer et al. 1998; Vandenbussche et al. 2004). Additionally, *Petunia* possesses two *PI* paralogs, *PhGLO1* and *PhGLO2*, that are the result of a recent duplication event (fig. 3*A*). Thus, the B gene complement of *Petunia* is double that of *Antirrhinum* or *Arabidopsis*. The retention of these paralogs has been accompanied by divergence in both biochemical and developmental aspects of gene function. The expression of *PhTM6* is restricted to the third and fourth whorls of the *Petunia* flower, and genetic evidence suggests that, unlike typical B-class genes, *PhTM6* is necessary for stamen development but does not seem to be involved in petal development (Vandenbussche et al. 2004; M. Vandenbussche and T. Gerats, personal communication). This shift in developmental function may represent a subfunctionalization (Force et al. 1999) or could reflect the original function of the ancestral paleo*AP3* lineage predating the eu*AP3/TM6* duplication (Lamb and Irish 2003; Vandenbussche et al. 2004). The recent *PI* paralogs also exhibit altered biochemical function in that PhGLO2 appears to prefer interacting with PhTM6 rather than PhDEF (Vandenbussche et al. 2004). These data suggest that the simple picture of B-class gene function as described for *Arabidopsis* and *Antirrhinum* is not strictly conserved, even across the eudicots, and they highlight the complex association between homologous structures and the genes underlying their development. Stamens in *Arabidopsis*, *Antirrhinum*, and *Petunia* are unquestionably homologous structures, but over the course of evolutionary time, their genetic control has experienced what is termed developmental system drift (DSD; True and Haag 2001). While stamen identity is controlled by two genes in *Arabidopsis* and *Antirrhinum*, in *Petunia* it is promoted by four genes, two ancient and two recent paralogs (fig. 3*B*). This situation is likely to be mirrored in the basal eudicot family Ranunculaceae (Kramer et al. 2003), as well as in other taxa that exhibit duplicate copies of *AP3* and/or *PI* (Tsai et al. 2004; Aagaard et al. 2005). As more comparative evidence becomes available across gene lineages, we will see additional cases of complex one-to-many and many-to-many relationships among important developmental loci. While such findings do not undermine the homology of deeply conserved structures such as stamens, they do indicate that simple homologous relationships among their developmental programs cannot be taken for granted.

Diversification of a Homologous Identity Program through Gene Duplication: Paralogous Genes and Homologous Morphology

Another case of gene duplication events that complicate comparisons among model species is that of the primary C-class genes in *Arabidopsis* and *Antirrhinum*, *AG* and *PLENA* (*PLE*), respectively. Both genes control the identity of the

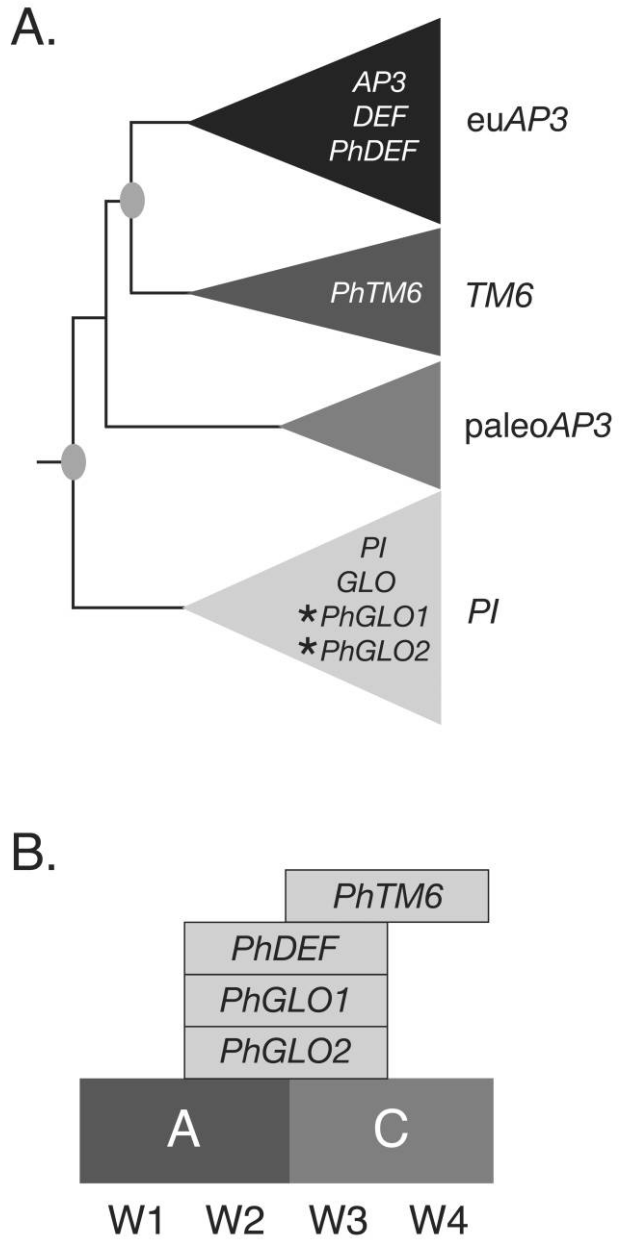

Fig. 3 *A*, Simplified phylogeny of the *AP3/PI* subfamily of MADS-box genes (Kramer et al. 1998; Stellari et al. 2004). Gray ovals indicate ancient duplication events. The *AP3* lineage is composed of three major sublineages known as eu*AP3*, *TM6*, and paleo*AP3*. The first two are restricted to the core eudicots, while the last is found outside the core eudicots. The *PI* lineage is shown as a single clade. The *Arabidopsis* and *Petunia* representatives of each lineage are shown (see text for references). Asterisks indicate paralogs due to recent duplication events. *B*, Modification of the ABC model for *Petunia*, with emphasis on the genes involved in B function. The model illustrates the floral whorls, numbered starting with the outermost: W1 (sepals), W2 (petals), W3 (stamens), and W4 (carpels). It should be noted that although *PhTM6* is expressed in the fourth whorl, it remains to be determined whether it plays a developmental role there.

fertile organs (stamens and carpels), as well as flower meristem determinacy (Bowman et al. 1989; Bradley et al. 1993). Similar to eu*AP3/TM6*, the *AG* lineage duplicated close to the base of the core eudicots to produce paralogous lineages that are represented by *AG* and *PLE* (fig. 4; Kramer et al. 2004; Causier et al. 2005). Therefore, these loci, previously treated as orthologs based on the inappropriate evidence of their functional and sequence similarity, are actually paralogous. *Arabidopsis* does possess orthologs of *PLE*, the recently duplicated *SHATTERPROOF1* and *SHATTERPROOF2* (*SHP1/2*) loci. These genes are responsible for specifying tissues that are unique to the silique fruit of the Brassicaceae (Liljegren et al. 2000) and promoting aspects of carpel and ovule identity, for which they are partially redundant with other *AG*-like genes (*AG* and *SEEDSTICK*; Favaro et al. 2003; Pinyopich et al. 2003). Likewise, *Antirrhinum* has an *AG* ortholog, the gene *FARINELLI* (*FAR*), but this locus primarily functions in stamen identity and development (Davies et al. 1999; Causier et al. 2005). *Petunia hybrida* has also retained orthologs of both gene lineages, *pMADS3* and *FBP6*, which contribute to reproductive organ identity and floral meristem determinacy (Kapoor et al. 2002). Thus, in each of these core eudicot model species, homologous fertile organs are produced by different combinations of paralogous members of the same MADS-box gene subfamily.

Given these findings, in addition to those regarding expression and function of basal *AG* homologs (reviewed in Kramer et al. 2004; Causier et al. 2005), the most parsimonious model is that the ancestral functional repertoire of the *AG* lineage encompassed stamen and carpel identity, floral meristem determinacy, and, most likely, some contribution to fruit and ovule development. Following the eu*AG/PLE* duplication event, these functions were independently divided between the two paralogous lineages during the diversification of the core eudicots. As a result, the paralogous *AG* and *PLE* inherited their similar functions from a common ancestor. This fact illustrates the point made by Theissen (2002) that orthology determinations do not rely on similarity of function but rather are a matter of phylogenetic relationships. Gene expression patterns, sequence similarity, and the capacity to complement mutant phenotypes are, likewise, not criteria for demonstrating orthology (Abouheif et al. 1997). Furthermore, it often requires increased sampling to fully understand phylogenetic relationships due to instances of gene loss (such as *TM6* in *Arabidopsis*) or intervening gene duplications (such as *PhGLO1/2* in *Petunia*).

Understanding the evolution of gene function within the context of gene lineage evolution can be difficult, and several authors have pointed out the problems inherent in the concept of functional homology (Bolker and Raff 1996; Abouheif et al. 1997). In morphological terms, the function of a structure cannot be considered homologous because it cannot be inherited, which is a fundamental criterion for homology assessment. From the genetic perspective, the argument has been made that because gene functions are in fact heritable, they can be shared due to common ancestry and therefore can be homologous (Mindell and Meyer 2001). One difficulty with this concept is that genetic functional repertoires tend to be highly changeable. Again, the *AG* subfamily can be taken as a good example. Overall, there does appear to be a conserved

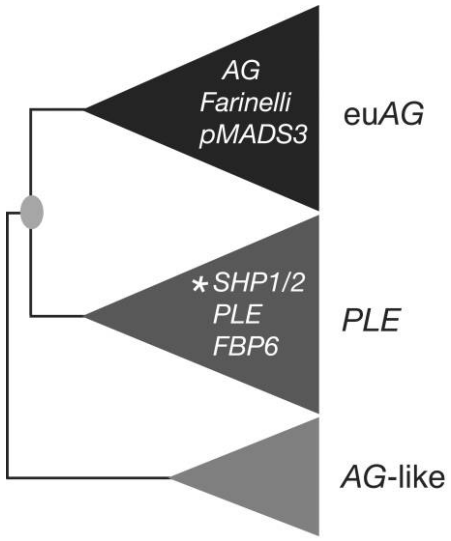

Fig. 4 Simplified phylogeny for the *AG* lineage (Kramer et al. 2004). Gray oval indicates the gene duplication event within the basal eudicots that gave rise to the core eudicot eu*AG* and *PLE* lineages. The *Arabidopsis*, *Antirrhinum*, and *Petunia* representatives of each lineage are shown (see text for references). Asterisk indicates a paralog due to recent duplication events.

functional repertoire across the subfamily. However, over the course of evolutionary time, this collection of functions has been differently parsed among the multiple *AG*-like paralogs. The result is a shifting pattern of functional evolution where new functions may be added just as others are lost. For instance, within the *AG* subfamily, there are multiple apparently independent derivations of paralogs with ovule-specific expression patterns (Kramer et al. 2004; Di Stilio et al. 2004). Although the majority of the functions represented by *AG*-like genes may be homologous in the sense of being derived via inheritance from a common ancestor, distinguishing where functional homology starts and ends is a difficult matter.

Serial Homology and Elaboration via Gene Co-Option

Plant lateral organs—leaves, sepals, petals, etc.—are serially homologous. Regardless of their identity, they share common genetic pathways that are responsible for producing a flattened lateral organ. This differentiation of upper (adaxial) and lower (abaxial) surfaces is due to the activity of specific sets of genes acting on each side of the organ (reviewed in Bowman et al. 2002). Adaxial identity is determined by members of the class III HD-ZIP transcription factor family, particularly *PHABULOSA* (*PHB*), *PHAVOLUTA* (*PHV*), and *REVOLUTA* (*REV*) (McConnell and Barton 1998; McConnell et al. 2001; Emery et al. 2003). Abaxial cell identity is determined by the combined and partially redundant action of members of the KANADI and YABBY families (Bowman and Smyth 1999; Sawa et al. 1999; Kerstetter et al. 2001). As with the MADS-box gene family, these loci have experienced multiple gene duplication events, resulting in patterns of genetic redundancy combined with novel functions. Of particular interest is the *Arabidopsis* YABBY gene *CRABS CLAW* (*CRC*), which functions in both carpel and nectary development (Alvarez and Smyth 1999; Bowman and Smyth 1999). Considering how these functions may have evolved within the *CRC* lineage provides some insight into the phenomenon of genetic co-option in plants. First, we examine *CRC*'s role in carpels, which most likely represents a specialization derived from the family's ancestral function in the development of lateral determinate organs. In *Arabidopsis*, *crc* mutants exhibit a slight loss of floral meristem determinacy and defects in stylar development (Alvarez and Smyth 1999; Bowman and Smyth 1999). This role in carpel development appears to be largely downstream of the multiple *AG* paralogs that promote carpel identity in *Arabidopsis* (Lee et al. 2005*a*). In contrast, the rice ortholog of *CRC*, a gene called *DROOPING LEAF* (*DL*), has much more severe affects on development (Yamaguchi et al. 2004). Analysis of several *dl* loss-of-function alleles demonstrates that *DL* is the primary promoter of carpel identity in rice and also plays a major role in floral meristem determinacy (Yamaguchi et al. 2004). These findings suggest that important aspects of C function have shifted not only among *AG* paralogs but also between unrelated transcription factors that participate in the same genetic pathway. Functional analyses of the *AG*-like genes in rice, two paralogs known as *OSMADS3* and *OSMADS58*, indicate that the function of *DL* in promoting carpel identity is independent of these loci, although *OSMADS58* also contributes to some aspects of carpel development (Yamaguchi et al. 2006). Again, these results in no way negate the homology of *Arabidopsis* and rice carpels but suggest that genetic contributions to their development vary.

Aside from this deeply conserved association with carpel development, both *CRC* in *Arabidopsis* and *DL* in rice exhibit what appear to be independently acquired functions. As mentioned above, *CRC* is important for nectary development in *Arabidopsis*. A recent comparative study of several core eudicot taxa indicates that this function is conserved not only across the Brassicales but also through the closely related Malvales and even the more distantly related Solanales (Lee et al. 2005*b*). These are very notable findings, given that the nectaries in question differ substantially in structure and position and had generally been considered to be independently derived (Fahn 1953, 1979). Analysis of the basal eudicot *Aquilegia*, however, does not show an association between *CRC* ortholog expression and nectary development (Lee et al. 2005*b*). This leads to the conclusion that *CRC* may have acquired an important role in secretory tissue specification before the diversification of the core eudicots. Of course, rice does not have nectaries, but *DL* does exhibit a unique expression domain, being localized to the developing midribs of vegetative leaves (Yamaguchi et al. 2004). This correlates with the drooping leaf phenotype of *dl* mutants, which lack proper midrib development. Furthermore, *DL* overexpression results in the ectopic formation of midrib-associated cells throughout the leaf blade. It remains to be determined whether this role in vegetative leaf development is generally conserved in other *CRC* orthologs or is perhaps characteristic of the distinct leaf type found in the grasses.

Overall, the diverse developmental roles exhibited by members of the YABBY family, both conserved and derived, exemplify how the high modularity of the angiosperm body plan has facilitated the co-option of genes and gene networks

for different roles in different organ types. Furthermore, we now know that genetic co-option can occur between structures that are not serially homologous. Most notably, the expression of meristem identity genes in diverse and independently derived compound leaves indicates that there may be a predisposition for certain co-option events (Bharathan et al. 2002). Perhaps the most surprising instance of genetic co-option to date is that of the *AP3* and *PI* paralogs of alfalfa, which appear to have been recruited to play a role in root nodulation (Heard and Dunn 1995; Heard et al. 1997). This example underscores the fact that genes such as *AP3* or *CRC* are just transcription factors and, ultimately, can be plugged into various genetic pathways to fulfill any conceivable developmental function.

Homology: Evidence from Identity versus Position

As authors begin to make comparisons across wider phylogenetic distances in the angiosperms, many studies become entangled in the distinction between correspondence of position and correspondence of identity. This is particularly true in regard to the evolution of the perianth and homologs of the *AP3* and *PI* gene lineages. The perianth takes an enormous diversity of forms and varies in organ number, whorl number, phyllotaxy, and differentiation of organs (fig. 5). Cladistic analyses indicate that early angiosperm lineages did possess petaloid organs that were most likely arranged in whorls, but whorl number is unclear, and differentiation into sepals and petals likely evolved several times independently (Zanis et al. 2003). In *Arabidopsis*, the establishment of petal identity relies on the expression of *AP3* and *PI* in the second-whorl floral primordia (Jack et al. 1992; Goto and Meyerowitz 1994), and, in consequence, *ap3* and *pi* mutants have sepaloid organs in place of petals (fig. 6*A*). Furthermore, these genes need to be expressed in every cell of the developing petal in order to confer petal identity (Jenik and Irish 2001). Studies across the angiosperms have repeatedly found a correlation between the expression of *AP3* and *PI* homologs and the development of petaloid organs. There are notable exceptions, however, two of which we consider in detail.

The first is that of the lodicule in grass flowers. Lodicules are nonpetaloid organs found in a position that appears to correspond to the second whorl of an *Arabidopsis* flower. The maize *AP3* and *PI* homologs, *SI1* and *ZMM16*, respectively, are expressed together in the lodicules and stamens (fig. 6*B*; Ambrose et al. 2000; Whipple et al. 2004). Functional studies of these loci and their rice orthologs demonstrate that they are required for lodicule and stamen identity in a manner consistent with B gene function (Kang et al. 1998; Ambrose et al. 2000; Nagasawa et al. 2003). In addition, like *AP3* and *PI*, *SI1* and *ZMM16* appear to function as obligate heterodimers and are able to bind to CArG boxes *in vitro* (Whipple et al. 2004). Consistent with these data, it has been found that *SI1* and *ZMM16* can largely substitute for *AP3* and *PI* function in *Arabidopsis* (Whipple et al. 2004). This type of experiment must be interpreted with great care, however. Heterologous expression essentially represents a kind of site-directed mutagenesis experiment that determines whether the sequence differences between, for example, *SI1* and *AP3*, reduce the ability of *SI1* to substitute

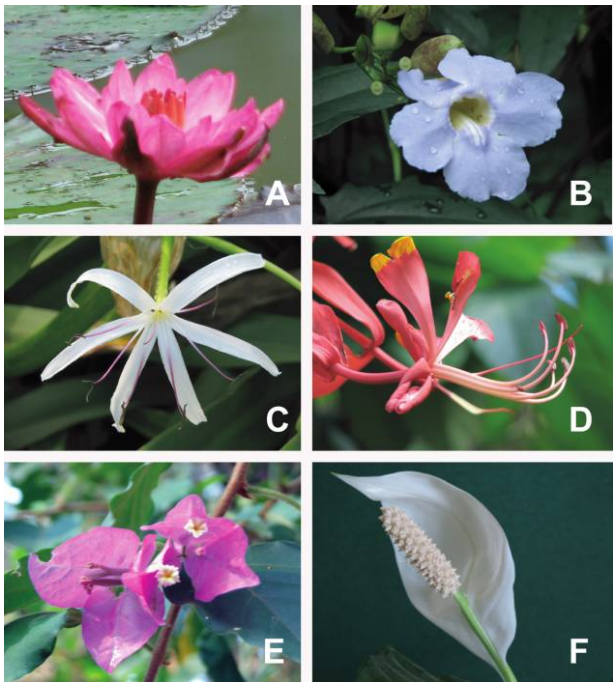

Fig. 5 Examples of diverse types of petaloid organs across the angiosperms. *A*, *Nymphaea rubra* (Nymphaeaceae), a member of one of the basalmost lineages of angiosperms, has several whorls of petaloid organs. *B*, *Thumbergia grandiflora* (Bignoniaceae), like many Asteridae, has a synpetalous corolla. *C*, *Crinum procerum* (Liliaceae), a monocot, and (*D*) *Amherstia nobilis* (Fabaceae), a eudicot, have petaloid organs in both first and second floral whorls. This condition is common in members of the Liliaceae but rare in the Fabaceae, with most species exhibiting a dichogamous perianth. *E*, *Bouganvillea spectabilis* (Nyctaginaceae), a eudicot, and (*F*) *Spathiphyllum walilisi* (Araceae), a monocot, have petaloid bracts subtending the inflorescences.

for *AP3* function in the genetic architecture of *Arabidopsis*. In the specific case of *SI1* and *AP3*, great care was taken to use the endogenous promoter, which is preferable to using a constitutive promoter. However, the transgenic lines analyzed contained seven to 11 copies of the trangenes and expressed two to five times the normal level of *AP3* transcript. This increased expression level may have affected protein interaction kinetics and would be expected to increase the level of what may have otherwise been poor protein interactions. These issues aside, it remains true that these levels of *SI1* and *ZMM16* were able to rescue many aspects of *AP3* and *PI* function. What is the significance of this finding? For one, it clearly underscores the sequence conservation between the homologous loci despite their considerable phylogenetic distance and multiple intervening gene duplications. This does not mean, however, that the downstream targets of *SI1* and *ZMM16* in their endogenous setting are the same as those of *AP3/PI* in *Arabidopsis*, particularly in regard to petal versus lodicule identity. It has been well established that the binding specificity of transcription factors is largely dependent on both their associated proteins and the sequence of the target promoters (reviewed in Wray 2003). The ability of the SI1/ZMM16 dimer to produce petaloid tissue is a product of the

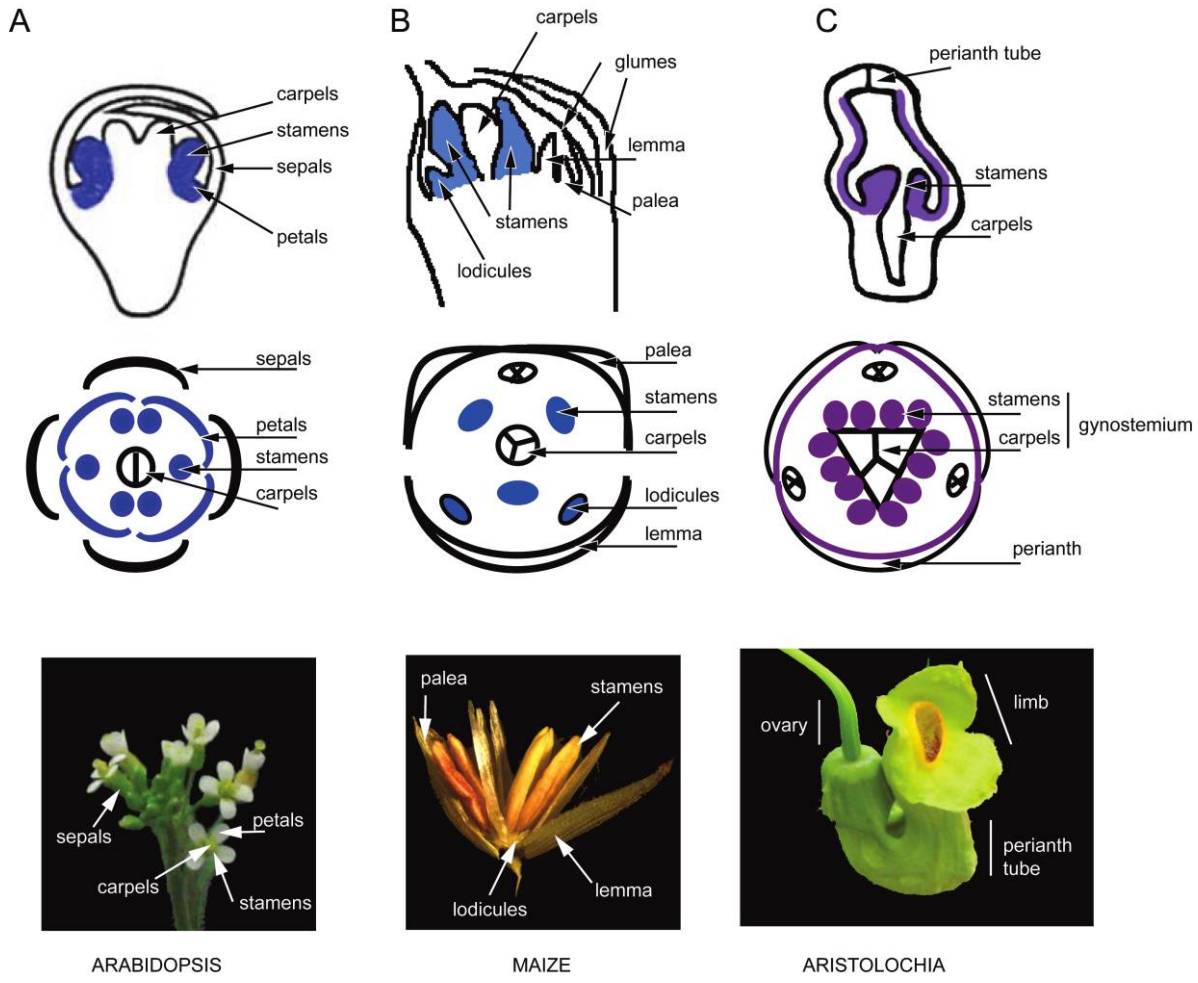

Fig. 6 Diagrams representing the floral morphology and *AP3/PI* homolog expression patterns in (*A*) the model eudicot *Arabidopsis* (Jack et al. 1992); (*B*) a model monocot, *Zea* (maize) (Ambrose et al. 2000; Whipple et al. 2004); and (C) a basal angiosperm, *Aristolochia manshuriensis* (Jaramillo and Kramer 2004). Drawings in the top row represent longitudinal sections of flowers, with the colored areas representing the expression domains of the *AP3* and *PI* homologs from each species. Floral diagrams in the middle row use color to indicate how these expression domains relate to the floral organs. In the bottom row, photos of each flower are provided for morphological comparisons.

Arabidopsis genome, which provides the cofactors that are expressed in the second whorl and the targets that possess appropriate CArG elements. In their natural setting, SI1 and ZMM16 are likely to interact with a distinct set of proteins and target loci, some of which are unique to the grass lodicule identity program. Given the rapid evolution of promoter sequences, there is little expectation that promoter elements and their associated target loci would remain invariantly conserved over long evolutionary time spans.

What has been clearly established is that *SI1* promotes lodicule identity, most likely in conjunction with *ZMM16*. It is also reasonable to conclude that lodicules are second-whorl organs (for morphological as well as molecular reasons) and that their identity program was derived from an ancestral petal identity program. This apparent transition from an ancestral second-whorl organ expressing a petal identity program to a similarly positioned organ possessing lodicule identity is analogous to the evolution of dipteran halteres from ancestral hindwings (reviewed in Gibson 1999). In that case, it has become clear that the *Ubx* gene is expressed in both butterfly hindwings and dipteran halteres, but there are significant differences in target gene regulation, despite high conservation in protein sequences. Likewise, when we consider the character of second-whorl organs, *Arabidopsis* petals and *Zea* lodicules may have positional correspondence, and they may further express identity programs that are derived from a commonly inherited pathway, but their end character states as petals and lodicules are morphologically and, most likely, genetically distinct. One interesting aspect of this scenario is that it remains to be determined how the second-whorl position of floral primordia is controlled independent of the organ identity genes. For example, functional dissection of the *AP3* promoter in *Arabidopsis* has shown that the petal-specific regulatory element is actually a whorl two-specific element that must be responding to whorl two-specific factors (Hill et al. 1998). It is possible that the common expression of *AP3* and *SI1* in second-whorl organs represents a deep conservation of this regulatory pathway (Kellogg 2004).

The second case we consider revolves around the issue of spatial deployment of identity programs, specifically in the magnoliid dicot *Aristolochia*. Unlike that of *Arabidopsis* or grass flowers, the *Aristolochia* perianth is composed solely of the calyx (first whorl) and is highly modified to form a trumpetlike structure that displays both petaloid and nonpetaloid characteristics. Petaloid characteristics, such as a bright color and a smooth epidermis, are restricted to the flower limb (fig. 6C). Conversely, the epidermis of the interior of the perianth tube has nonpetaloid characteristics, including trichomes and stomata (Jaramillo and Kramer 2004). The *AP3* and *PI* homologs of *Aristolochia manshuriensis* are expressed in the first-whorl calyx, but the expression is restricted to the nonpetaloid tissue of the perianth tube (Jaramillo and Kramer 2004). Therefore, this is a situation in which, arguably, petaloid first-whorl organs express *AP3/PI* homologs but do not appear to use these genes in the production of petaloid tissue. Just as the lodicule study demonstrated that *AP3/PI* can produce nonpetaloid sterile organs, the *Aristolochia* results demonstrate that petaloid tissue can develop without the direct contribution of these genes. Under the terminology of Baum and Donoghue (2002), this would qualify as nonhomologous transference of function: the calyx has taken over attractive functions from the corolla, but this was not accomplished through homeosis. Furthermore, when considered in combination with additional studies that have documented apparent shifts of *AP3/PI* expression into the first-whorl petaloid organs of monocots and members of the basal eudicots (Kanno et al. 2003; Kramer et al. 2003; Ochiai et al. 2004; Tsai et al. 2004; Nakamura et al. 2005), these findings underscore the mobile nature of organ identity programs (Baum and Donoghue 2002). Of course, the fact that *AP3/PI* homologs contribute to the development and/or identity of first-whorl organs does not mean that these organs are homologous to *Arabidopsis* petals. While such structures may express an identity program related to that expressed in the second whorl of *Arabidopsis*, their position is more likely to correspond with that of the *Arabidopsis* first whorl. There are several analogous cases of spatial shifts in identity from animal comparative studies, perhaps the most notable being the expression of *Hox* genes within the axial skeleton of vertebrates (Burke et al. 1995). This study found that the transposition of *Hox* gene expression was correlated with homeotic shifts of morphological domains along the anterior-posterior axis of diverse vertebrates. Similar to the situation in plants, this phenomenon produces a decoupling of position from the morphological similarity of the structures in question.

The above examples help to show the importance of recognizing the different hierarchical levels of homology and the occurrence of dissociability between the position of organs and the expression of specific genetic identity programs. In the case of petals, the distinction is complicated because the term refers to both position and character state. The second-whorl organs are usually petaloid and seem to commonly express *AP3/PI* homologs (reviewed in Kramer and Irish 2000; Kim et al. 2005*b*; Zahn et al. 2005), but petaloid organs are not always in the second whorl of flowers and may not always express B gene homologs. Further research is urgently needed to understand how position is controlled at a genetic level. Hopefully, this will lead to a better understanding of the dissociation between the position of an organ and the homology of the identity program it expresses.

Final Comments and Guidelines for Using Gene Expression Data to Understand Homology

There are a number of take-home lessons that can be derived from the examples discussed above, but we would like to start with the most practical. Specifically, it is important to note the utility of thorough analyses of gene lineage evolution combined with detailed examination of gene expression using *in situ* hybridization and functional studies in original taxa. A good understanding of gene lineage evolution through phylogenetic analyses of a broad sample of taxa is crucial to determine the orthology relationships among genes. The C-class genes *AG* and *PLE* were long considered orthologous based on functional and sequence similarity, but increased phylogenetic sampling and genomic analyses have shown otherwise. *In situ* hybridization analyses are also important because there are drawbacks to relying solely on results from reverse transcriptase PCR (RT-PCR) and Northern hybridization. In contrast to the latter two techniques, *in situ* hybridization does not depend on the ability to dissect specific organs and thereby allows the analysis of early developmental stages as well as organs that are fused. In addition, *in situ* hybridization has the capacity to show spatial and temporal differences in expression patterns that are unlikely to be identified using other types of comparative expression approaches. For instance, RT-PCR on the organs of *Aristolochia* simply detects *AP3/PI* expression in both the perianth and the fused gynostemium, while *in situ* hybridization reveals the actual complexity of the genes' expression. Finally, functional analyses are key to the understanding of gene activity because conservation of expression pattern may not represent strict conservation of function (Baum 2002; Baum et al. 2002; see also David-Schwartz and Sinha 2007). This fact is underscored by the work with *DL* in *Oryza*. On its own, the expression of *DL* in carpels would likely lead to a conclusion that the locus functions in a manner similar to *CRC*. Even heterologous complementation experiments would be unlikely to reveal *DL*'s critical role because this is almost certainly a product of *Oryza*'s genomic architecture rather than a result of inherent differences in the protein itself.

The above examples clearly illustrate the difficulties inherent in using expression analyses to assess structural homology. However, it is also apparent that the study of developmental genetics has added a great deal to our understanding of morphological evolution. In order to make comparative expression analyses more meaningful, we need to take into account (1) the evolutionary history of the morphological feature in question through reconstructions on robust phylogenies; (2) the evolutionary history of the candidate gene, because gene duplications are very common (ca. 90% of *Arabidopsis* and 62% of the rice genome are duplicates; Moore and Purugganan 2003) and these events may make the occurrence of neo- and subfunctionalization more common among plants than other organisms; (3) a clear definition of the morphological character of interest; in the case of flowers—perhaps the best-understood angiosperm structures—it is important to distinguish each whorl of organs as a distinct character and the identity that

they can take (sepaloid organ, petaloid organ, stamen, carpel) as a character state; (4) comparative expression data from multiple genes in a genetic network responsible for the character of interest (Abouheif 1999). At present, most comparative expression analyses focus on the genes in the ABC model; however, little is known about regulators and targets of these organ identity genes. These guidelines suggest several lines for future research. It would be particularly useful to focus comparative expression and functional analyses on closely related taxa, where heterologous transformations would be more meaningful and intermediate morphologies are often available (Baum 2002). In addition, detailed studies of intermediate taxa that span the phylogenetic distance between the better-studied *Arabidopsis* and rice, for example, basal angiosperms and lower eudicots, are key to understanding the angiosperms' ancestral genetic tool kit. The development of new model species within these groups is, therefore, very important.

Finally, we would like to return to the definition of homology cited in the introduction to this article: "correspondence caused by a continuity of information" (Van Valen 1982), with continuity being provided by common descent and the information in question being heritable and, therefore, fundamentally genetic in nature. We have argued, however, that genetic information is not always a reliable indicator of homology, especially when only one gene is examined. Our particular concern is the conflation of positional evidence for homology with evidence from identity. Many of the genes that have been studied comparatively are floral organ identity homologs, and it appears that such identity programs can be shifted spatially, resulting in homeosis. Thus, the activity of, for example, an *AP3* homolog in a particular organ should be taken primarily as an indicator of the identity program being expressed rather than the organ's position. Obviously, there must also be genetic information that controls position, but this is not well understood at the present. It is also possible that as we obtain more information on expression dynamics, it will become clear that there are, in fact, qualitative differences in the ways that these genes are expressed in different whorls (Kramer 2005; Kramer and Jaramillo 2005).

This argument is consistent with Gilbert and Bolker's (2001) distinction between process homology and structural homology, which represent different hierarchical levels of homology. Process homology reflects the common inheritance of developmental genetic pathways or modules that can be co-opted to function in diverse situations. For this reason, process homology is dissociable from structural homology and is often invoked in cases where different hierarchical levels of homology lack straightforward correspondence (Mindell and Meyer 2001; Brigandt 2003). As proposed by Baum and Donoghue (2002), novel combinations of such genetic modules may be at the heart of many of Sattler's (1984, 1994) examples of partial homology or may promote the evolution of novel organ identities (Kramer and Jaramillo 2005). However, such modules are not always strictly conserved due to sub- and neofunctionalization as well as other sources of DSD. As we learn more about developmental pathways as a whole, including gene targets and regulators, we may discover that these master organ identity genes changed their main function during the course of evolution, taking on several roles within the genetic modules. Ultimately, therefore, our assessments of process homology will be more robust if they are derived from comparisons of whole genetic networks (Abouheif 1997, 1999). In general, when discussing comparative studies of organ identity genes, it is important to be terminologically explicit that resultant data are an assessment of process homology (or homocracy; Nielsen and Martinez 2003) and may not translate directly into the structural homology of the organ in question. Of course, other aspects of morphological homology, such as exact positional correspondence, can be equally difficult to determine, especially when comparisons are made across large phylogenetic distances. For instance, the petaloid tepals of *Amborella* may have process homology with the petals of *Arabidopsis* (Kim et al. 2005*b*), but their specific positional relationship is harder to evaluate (aside from generally being outside of the fertile organs). As Tautz (1998, p. 17) observed, homology, like the Hardy-Weinberg equilibrium, is "an idealized principle that works under idealized conditions, but such conditions almost never apply." Despite this lament, we do believe in the continued utility of the concept, as long as it is applied with care and specificity.

Acknowledgments

We would like to thank Jocelyn Hall, Barbara Gravendeel, Amy Litt, and Sarah Mathews for constructive conversations on the topic of homology in plants. We would also like to thank two anonymous reviewers for comments on the manuscript. Work described here was supported by the Mercer Fellowship of the Arnold Arboretum to M. A. Jaramillo and National Science Foundation awards EF-0412727 and IBN-03139103 to E. M. Kramer.

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